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Heal War Wounds

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13. ABSTRACT (Maximum 200 Words) Wound healing is a controlled coordinated response to tissue injury leading to scar tissue formation. It involves complex interactions between cell types, matrix and soluble mediators. When one or more of these components is disrupted, wound healing is delayed or even halted. There has been some success with topical application of growth factors to improve healing in the face of sepsis and infection. However the effects have been modest. A major limitation of topical application of growth factors is that they are destroyed rapidly by tissue proteases. The gene therapy approach we propose will have the advantage of continually producing growth factors within the wound to constantly replenish tissue levels. Our approach also allows the production of multiple growth factors to maximize potential benefit.				
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Introduction.

Impaired healing of war wounds remains an unsolved military medical problem. It involves complex interactions between cell types, matrix and soluble mediators. In sepsis which is often associated with war wounds, one or more of these components is disrupted. One of the key elements in wound repair is the release of growth factors (e.g. EGF, PDGF, FGF-1, VEGF, KGF-1), which bind to specific receptors on the target cell and activate signal transduction pathways that finally result in proliferation, migration or synthesis of structural elements.

There has been some success with topical application of growth factors to improve healing in the face of sepsis and infection (2-5). However the effects have been modest. A major limitation of topical application of growth factors is that they are destroyed rapidly by tissue proteases. The gene therapy approach we propose will have the advantage of continually producing growth factors within the wound to constantly replenish tissue levels. We are testing this approach using electroporation to deliver growth factor plasmid DNA to a rodent war wound model.

Body

Describe the research accomplishments associated with each task outlined in the approved Statement of Work.

Year 1.

- 1) Voltage of 1800 was required to increase the transfection efficiency of the luciferase reporter gene into the rat model and that a single application of plasmid was just as effective as a double dose of plasmid. (see appended manuscript fig 3 and fig 4.)
- 2) E.coli impregnated agar pellets and cecal ligation to model sepsis was changed to model with cecal ligation only because of high mortality rate in previous model. (previous manuscript??)
- 3) Using KGF-1 plasmid DNA faster wound closer was noted than control, however burst strength was unchanged (previous manuscript??)

Year 2.

- 1) Wound histology reveals improved wound quality with KGF-1 plasmid DNA gene therapy (see appended manuscript Fig. 9 and Fig 10)
- 2) Using luciferase reporter plasmid DNA injected intradermally shows gene expression predominately in the rat epithelium. (see appended manuscript Fig. 6 and Fig 7)
- 3) Electroporation increases transfection efficiency 53 fold as compared to unelectroporated controls. (see appended manuscript Fig 2.)
- 4) Multiple growth factors do not improve wound closure as compared to KGF-1 alone.
- 5) Multiple growth factors do not effect collagen levels in final wound biopsy

Key Research Accomplishments

- 1) Voltage of 1800 was required to increase the transfection efficiency of the luciferase reporter gene into the rat model and that a single application of plasmid was just as effective as a double dose of plasmid. (see appended manuscript fig 3 and fig 4.)
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- 4) Wound histology reveals improved wound quality with KGF-1 plasmid DNA gene therapy
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- 7) Multiple growth factors do not effect collagen levels in final wound biopsy

Reportable Outcomes.

Brynes CK, Malone RW, Akhter N, Hilal S, Nass PH, Wetterwald A, Cecchini MG, Duncan MD, Harmon JW. Electroporation enhances transfection efficiency in murine cutaneous wounds. *Wound Rep Reg* 2004; 12: 397-403.

Lin MP, Marti GP, Dieb R, Wang J, Qaiser R, Bonde P, Duncan MD, Harmon JW. Electroporation Improves transfection efficiency in rat wound healing model. *J Am College of Surgeons* 199:S58, 2004.

Marti GP, Lin, MP, Qaiser R, Dieb R, Wang J, Parth S, Bonde P, Duncan MD, Harmon JW. KGF-1 plasmid delivered with electroporation accelerates wound closure in diabetic mice. *J Am College of Surgeons* 199:S59, 2004.

Marti G, Ferguson M, Wang J, Dieb R, Qaiser R, Bonde P, Duncan MD, Harmon JW. Electroporative transfection with KGF-1 DNA improves wound healing in a diabetic mouse model. *Gene Therapy* 2004 0, 1-6.)

Mark Ferguson Ph.D., Colman Brynes MB ChB¹, Eric Sun², Guy Marti MD, Pramod Bonde MD, Mark Duncan MD, John W Harmon MD. Wound healing enhancement: Electroporation to address a classic problem of military medicine. Submitted *World Journal of Surgery*. In Review 2004

Translational Research Accomplishments:

Establishment of Company to develop the technology:

We incorporated Canton Biotechnologies Inc in the State of Maryland. We have been granted a State of Maryland Biotech Incubator grant from TEDCO for the development of the company and the technology. This is a \$50,000 grant and we are applying for a second grant. We have assembled a strong Board of Directors and have access to Angel investors at this time.

We are licensing our Electroporation-Gene Therapy patent from Johns Hopkins University. We (JWH, PI) applied for this patent prior to obtaining the DOD funding based on our preliminary work. But the Army grant has been critical to our success in moving this project forward.

We would be very interested in a DOD SBIR grant to allow Canton Biotechnologies Inc to bring this promising technology to the clinical arena.

Conclusions

We have received approval for a NCE. The period of work will now end Jan, 2006. In that period we will complete our experiments as outlined in our proposal. We will also complete and submit a manuscript describing the results of the studies in detail. At this point we have presented our early findings already at the annual meeting of the American College of Surgeons at the Surgical Forum in October 2004.